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SUPERIOR DEVELOPMENTAL STABILITY OF HETEROZYGOTES AT ENZYME LOCI IN SALMONID FISHES

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Waddington (1942, 1948) was one of the first authors to stress the importance of the developmental process in evolution from a genetics perspective. He proposed that natural selection acts on development primarily to produce pathways that are insensitive to minor changes in the environment and the genome so that the phenotypic norm of the species will result (Waddington 1942). He referred to this process as the buffering or canalization of development. This "canalizing selection" on development ensures the relative constancy of the phenotype through evolutionary time (Waddington 1960; Waddington and Robertson 1966). Alterations in developmental pathways, and thus the phenotypic norm, require either changes in selection pressures, so that the norm is no longer associated with maximum fitness, or the presence of new genetic variability capable of having a phenotypic effect despite canalization. Waddington (1942) emphasized the importance of dominance and dosage compensation as genetic mechanisms producing canalization.

Lerner (1954) extended these ideas by suggesting that heterozygosity acts to stabilize development. That is, heterozygosity confers an increased ability to compensate for both environmental and genetic variation during development so that the genetically determined developmental pathways are more precisely expressed in the phenotype of individuals. Consequently, he predicts that more heterozygous individuals will have increased developmental stability and be closer to the phenotypic norm.

Increasing emphasis is being placed on the importance of the developmental process and its genetic regulation to bring about evolutionary change (Britten and Davidson 1969; Frazetta 1970; Wilson 1976; Gould 1980). Most studies have focused on regulatory loci and their potential to produce large organismal effects of possible adaptive value (MacIntyre 1982; Allendorf et al. 1983; Leary et al. 1984). These studies have increased our knowledge of what types of genetic variation are capable of producing phenotypic change regardless of any developmental canalization.

Several recent studies have attempted to determine the genetic mechanisms responsible for developmental canalization (see Soulé and Cuzin-Roudy 1982 and Leary et al. 1983*a* for a review). Fluctuating asymmetry of bilateral traits has been

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used as a measure of developmental stability in many of these studies because it is considered to reflect the inability of an organism to develop precisely along determined pathways (Mather 1953; Lerner 1954; Thoday 1958; Van Valen 1962; Soulé 1967; Felley 1980). Fluctuating asymmetry is defined to occur when the difference between a character on the left and right sides of individuals is normally distributed about a mean of zero (Van Valen 1962). Increased developmental stability is reflected by reduced amounts of fluctuating asymmetry.

We have previously shown a significant negative correlation between enzyme heterozygosity at 42 loci and fluctuating asymmetry at five bilateral characters among individuals in a population of rainbow trout (*Salmo gairdneri*) (Leary et al. 1983*a*). In this paper, we extend this observation to other domestic and natural populations of rainbow trout, and to natural populations of westslope cutthroat trout (*Salmo clarki lewisi*) and brook trout (*Salvelinus fontinalis*). The results demonstrate that more heterozygous individuals have reduced fluctuating asymmetry and that this appears to be general among salmonid fishes.

Fluctuating asymmetry has been assumed to be a reliable measure of developmental stability and to be positively associated with fitness (Soulé 1982; Soulé and Cuzin-Roudy 1982). We present data in this paper that lend support to these assumptions. Thus, heterozygosity at isozyme loci, or the chromosomal segments marked by these loci, is apparently of adaptive significance, suggesting that overdominant selection may play an important role in maintaining the genetic variation detected in these species.

METHODS

Sample preparation and electrophoresis in starch gels followed Utter et al. (1974) with the stains and buffer systems of Allendorf et al. (1977). Isozyme loci are designated using the nomenclature described by Allendorf et al. (1983). The following enzymes encoding 42 loci were screened: adenylate kinase (ADK; EC 2.7.4.3), alcohol dehydrogenase (ADH; 1.1.1.1), aspartate aminotransferase (AAT; EC 2.6.1.1), creatine kinase (CK; EC 2.7.3.2), glucosephosphate isomerase (GPI; EC 5.3.1.9), glyceraldehyde-3-phosphate dehydrogenase (GAP; EC 1.2.1.12), glycerol-3-phosphate dehydrogenase (G3P; EC 1.1.1.8), glycyl-leucine peptidase (GL; EC 3.4.11), isocitrate dehydrogenase (IDH; EC 1.1.1.42), lactate dehydrogenase (LDH; EC 1.1.1.27), leucyl-glycyl-glycine peptidase (LGG; EC 3.4.11), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.40), phosphoglucomutase (PGM; EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6PG; EC 1.1.1.44), sorbitol dehydrogenase (SDH; EC 1.1.1.14), superoxide dismutase (SOD; EC 1.15.1.1), and xanthine dehydrogenase (XDH; EC 1.2.3.2).

Salmonid fishes are derived from a tetraploid ancestor of some 25 to 100 million years ago (Ohno 1974). Because of the tetraploid ancestry some loci are functionally duplicated. That is, some pairs show no evidence of either structural or regulatory divergence (Bailey et al. 1970). We treated these pairs as a single functional locus and classified "genotypes" the same as in Leary et al. (1983*a*).

Fish were obtained from the following populations, with the sample sizes in

parentheses: domestic rainbow trout: Arlee-1 (50), Arlee-2 (300), Boulder (57), Chambers Creek (60), Erwin (60), Goldendale (25), McConaughy (65), Shasta (66), and Tasmanian (49); natural rainbow trout: Sinker Creek, Owyhee County, Idaho (51); natural westslope cutthroat trout: Granite Creek, Missoula County, Montana (27), and O'Keefe Creek, Missoula County, Montana (51); natural brook trout: Mud Lake, Lake County, Montana (28), and Tin Cup Creek, Ravalli County, Montana (59). The domestic rainbow trout were obtained from the Montana Department of Fish, Wildlife and Parks (Arlee), the Washington State Department of Game (Chambers Creek and Goldendale), and the United States Fish and Wildlife Service (the remaining strains).

In addition, two other domestic populations that contain a high frequency of individuals with obvious morphological deformities (phenodeviants) were sampled. The Goldendale × Packwood Lake rainbow trout population was founded by the Washington State Department of Game in 1982 by crossing a single female from the Goldendale Hatchery with four males from a natural population in Packwood Lake, Washington. This hybrid population contains only 80% of the electrophoretically detectable heterozygosity in the Goldendale population. The history of the Murray Springs westslope cutthroat trout population is reviewed by Allendorf and Phelps (1980). This population contains only 75% of the heterozygosity present in the natural population from which it was derived.

RESULTS

Heterozygosity and Asymmetry

The counts of five bilateral meristic characters that exhibit fluctuating asymmetry in all the samples were taken on the left and right side of each fish: rays in the pelvic fins, rays in the pectoral fins, gillrakers on the lower first branchial arches, gillrakers on the upper first branchial arches, and mandibular pores. Phenotypes were scored at 42 isozyme loci using starch gel electrophoresis. The results of correlation analyses between the proportion of heterozygous loci and asymmetric characters per individual are summarized in table 1. In two of the domestic rainbow trout populations (Arlee and Goldendale), and in both of the natural westslope cutthroat trout populations, there is a significant negative correlation between these values. Furthermore, the sign of the correlation coefficient is negative in 13 of the 14 analyses (sign test, $P < .001$). The results using the magnitude of asymmetry (Leary et al. 1983*a*) are qualitatively the same as those above and thus we do not present them in detail.

The Arlee-1 and Arlee-2 samples are two generations of fish from the same hatchery strain. The Arlee-2 fish are the progeny from 12 full-sib families (25 fish each) created by mating 24 of the 50 fish from the Arlee-1 sample. Thus, two separate samples from this strain show a significant negative correlation between heterozygosity and asymmetry.

We next examined whether this negative correlation between heterozygosity and fluctuating asymmetry in these populations is associated with individual loci or a general heterozygous effect. The distributions of both measures of asym-

TABLE 1

SUMMARY OF CORRELATIONS BETWEEN INDIVIDUAL HETEROZYGOSITY AND ASYMMETRY IN THREE SPECIES OF SALMONID FISHES

Species	Sample	Heterozygosity (%)	Mean Asymmetry	Correlation
Brook trout	Mud Lake	7.4	1.43	−.01
	Tin Cup Creek	5.1	1.59	−.13
Rainbow trout	Arlee-1	6.5	1.80	−.40**
	Arlee-2	7.7	1.46	−.12*
	Boulder	8.5	1.37	−.11
	Chambers Creek	5.4	1.35	−.11
	Erwin	5.2	1.60	−.13
	Goldendale	8.4	1.60	−.41*
	McConaughy	6.3	1.43	.21
	Shasta	8.5	1.89	−.01
	Sinker Creek	5.5	1.61	−.06
	Tasmanian	7.7	1.74	−.07
Cutthroat trout	Granite Creek	3.8	1.86	−.46**
	O'Keefe Creek	3.9	1.82	−.25*

* $P < .05$.
** $P < .01$.

metry per individual were compared between homozygotes and heterozygotes in each population with the Wilcoxon two-sample test. Homozygotes for the common and variant alleles at individual loci were not found to differ for the two measures of asymmetry so we included both in the homozygous category. We used only those loci in which the common allele occurs in a frequency less than 0.95.

There is some indication that heterozygosity at *Sod1* in rainbow trout and *Aat3,4* in brook trout may have a greater effect on fluctuating asymmetry than heterozygosity at other loci. Heterozygotes have significantly ($P < .05$) less asymmetry in only 6 of the 70 total comparisons in rainbow trout (table 2). Half of these significant differences are at *Sod1*. There is also a significant excess of populations in which heterozygotes at *Sod1* in rainbow trout have a lower mean proportion of asymmetric characters than do homozygotes (8 and 2, sign test, $P = .04$). *Aat3,4* heterozygotes in brook trout have significantly less asymmetry than homozygotes in both populations (table 2). Heterozygosity at these loci alone, however, is not sufficient to account for the association between heterozygosity and fluctuating asymmetry that we have observed in these three species. Thus, this association appears to be due to a general heterozygous effect, although a few loci may have a greater effect than the others.

The positive correlation coefficient in the McConaughy rainbow trout strain (table 1) warrants some discussion. This strain, sampled during its second generation of domestication, was founded from a natural population that was recently created by the introduction of numerous rainbow trout strains into Lake McConaughy, Nebraska. This strain, unlike all the other populations we sampled, contains genetic material from a number of different populations that have only

TABLE 2

DIFFERENCES IN MEAN ASYMMETRY BETWEEN HOMOZYGOTES AND HETEROZYGOTES AT TWENTY-FOUR ENZYME LOCI IN POPULATIONS OF THREE SALMONID SPECIES

	RAINBOW TROUT		CUTTHROAT TROUT		BROOK TROUT	
LOCUS	+	−	+	−	+	−
Aat3,4	2	0	1	1	2(2)	0
Ckl	1	0	0	0	0	0
Gap4	0	0	1(1)	0	0	0
G3pl	2	2	0	0	1	0
Gll	1	1(1)	0	0	0	0
Gpil	0	0	1	0	0	0
Gpi3	0	0	0	0	0	1(1)
Idh2	5	3	0	0	0	0
Idh3,4	3(1)	6(1)	1(1)	1	0	1
Ldhl	0	0	1	0	0	0
Ldh3	0	0	0	0	0	2
Ldh4	3	1	0	0	0	0
Ldh5	2(1)	2	0	0	0	0
Lgg	2	0	0	0	0	0
Mdh3,4	7	2	0	0	0	1
Mel	1	1	0	0	1(1)	0
Me4	2(1)	1	0	0	1	0
Pgml	0	0	1	0	0	0
Pgm2	4	1(1)	0	0	0	0
Sdh	4	1	0	0	0	0
Sodl	8(3)	2	1	0	0	0
Totals	47(6)	23(3)	7(2)	2	5(3)	5(1)

NOTES.—The values presented are the number of populations in which the mean number of asymmetric traits per individual for homozygotes minus this value for heterozygotes has the indicated sign. Numbers in parentheses represent the number of populations in which the homozygotes and heterozygotes are significantly different ($P < .05$).

recently been interbreeding. The positive sign of the correlation in this population might indicate a lack of integration of these different genomes in the control of the developmental process. This view is currently purely speculative. We are, however, collecting data from artificially produced salmonid hybrids and from naturally introgressed populations that are directed at this issue.

Asymmetry and Developmental Stability

Although fluctuating asymmetry is generally assumed to be positively associated with developmental stability and fitness, there are little data upon which to judge the validity of this view (Soulé 1982). We compared the distributions of asymmetry per individual between those fish with and without obvious morphological deformities in three domestic populations: Goldendale × Packwood Lake rainbow trout, truncated upper jaw; Shasta rainbow trout, extra pelvic fins; and Murray Springs westslope cutthroat trout, missing pectoral fins, incomplete vertebral column, and fish with both deformities. If fluctuating asymmetry is a good measure of overall developmental stability, then phenodeviants should also have increased asymmetry.

TABLE 3

COMPARISON OF NUMBER OF ASYMMETRIC TRAITS BETWEEN NORMAL AND DEFORMED RAINBOW TROUT AND WESTSLOPE CUTTHROAT TROUT

	Sample Size		Mean Asymmetry		
Species-Samples	Normal	Deformed	Normal	Deformed	Probability
Rainbow trout					
Goldendale × Packwood	30	14	1.53	2.29	<.001
Shasta	60	6	1.73	3.33	<.001
Cutthroat trout					
Murray Springs					
normal vs. one deformity	24	48	1.50	2.00	<.05
normal vs. two deformities	24	26	1.50	2.62	<.001
one vs. two deformities	48	26	2.00	2.62	<.001

NOTES.—The number of asymmetric characters in the Murray Springs data is based only on four characters because 52 of the 98 fish in this sample were missing one or both pectoral fins. Probabilities based on Wilcoxon two-sample test.

The results of these comparisons using the number of asymmetric characters per individual are summarized in table 3. In all cases the normal fish have a significantly lower distribution of the number of asymmetric characters than the deformed fish. Furthermore, in the Murray Springs sample the fish with only one deformity have a significantly lower distribution of asymmetric characters than those with two. We obtained the same results for all comparisons using the magnitude of asymmetry (fig. 1).

DISCUSSION

Heterozygosity and Asymmetry

The evidence supporting the view that heterozygotes are more developmentally stable has been criticized recently (Clarke 1979; Chakraborty and Ryman 1983). The original evidence came from lower variance of metrical characters in hybrids. However, heterosis between blocks of genes can also result from dominance at individual loci (Clarke 1979). Several authors have found that populations with higher heterozygosity at loci encoding enzymes tend to have significantly less fluctuating asymmetry (Soulé 1979; Kat 1982; Vrijenhoek and Lerman 1982). Such interpopulation correlations can be caused by other factors and thus do not provide evidence that more heterozygous individuals within random mating populations are more developmentally stable. Other authors have reported that heterozygotes have less phenotypic variability for meristic and morphometric characters (Mitton 1978; Eanes 1978). This relationship, however, can be accounted for by simple additive genetic variation controlling these characters (Chakraborty and Ryman 1983).

Ours are the first results we are aware of that show an association between heterozygosity and developmental stability between individuals within random mating populations. This association apparently results from heterozygosity at many loci with small effects spread throughout the genome. None of the poly-

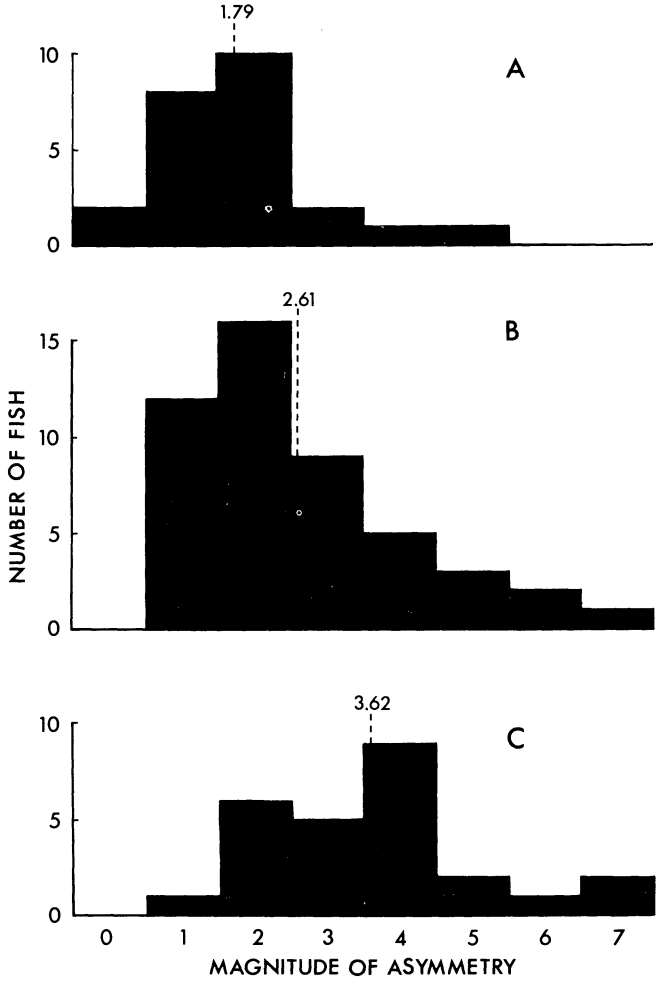

FIG. 1.—Distribution of the magnitude of asymmetry in normal fish (A), fish with one deformity (B), and fish with two deformities (C) in cutthroat trout from the Murray Springs Hatchery.

morphic loci that we detected in rainbow and brook trout are known to be linked (May et al. 1982). Linkage data are not available for westslope cutthroat trout but linkage relationships in salmonids appear to be conserved (May et al. 1982).

The finding that obviously deformed fish are more asymmetric indicates that fluctuating asymmetry is apparently a good indicator of overall developmental stability. These results also suggest that fluctuating asymmetry is negatively associated with fitness since these deformities would adversely affect the survival of individuals in natural situations. The jaw deformity would undoubtedly reduce the ability of these carnivorous fishes to obtain food. The paired fins in fishes are used largely for maintaining an upright position and balance during swimming, for vertical movement in the water column, for maintaining a stationary position, and

for the cessation of movement. The presence of extra or absence of some of these structures would undoubtedly adversely affect the ability of individuals to perform these functions important in maintaining territories, acquiring food, and escaping predation. Similar results showing an association between dental asymmetry and morphological deformities have been reported in humans (Bailit et al. 1970).

Possible Mechanisms

Recent papers have stressed the difficulty of detecting a phenotypic effect of heterozygosity through an examination of the relatively small proportion of the genome amenable to electrophoretic analysis (Chakraborty 1981; Mitton and Pierce 1980). Nevertheless, a relationship between heterozygosity and reduced asymmetry is present in our data and it must somehow be explained.

Haldane (1954, p. 121) was apparently the first to suggest that having two different forms of the same enzyme may have a buffering effect on the phenotype by maintaining a more constant metabolic flux under varying conditions (e.g, temperature or substrate concentration). Several more recent papers have extended Haldane's suggestions (e.g., Fincham 1972; Berger 1976). Although attractive, the evidence supporting this view has remained sparse (Clarke 1979).

A second possibility is that tightly linked cis-acting regulators may be responsible for phenotypic effects associated with variation at structural loci encoding enzymes (Wallace 1976). Cis-acting regulators linked to enzyme loci have been found to be common in eukaryotes (Dickinson 1983). Such regulatory elements determine the time and place during which structural loci encoding enyzmes are expressed (Paigen 1979). The element responsible for the expression of a structural locus is usually dominant over the element associated with nonexpression (Paigen 1979).

It is likely that variation at such regulators would be in linkage disequilibrium (gametic phase imbalance) with variation at the corresponding structural gene because of tight linkage. Thus, heterozygotes at a structural locus are also more likely to be heterozygous at a cis-linked regulator. Recent studies (see MacIntyre 1982 for review) have shown that regulatory variation can have important effects, including increased developmental stability (Allendorf et al. 1983). Therefore, increased heterozygosity at regulatory genes may be responsible for the association between heterozygosity at enzyme encoding loci and developmental stability.

There is a third possible explanation for the association between heterozygosity and asymmetry. The observed effects may be caused by increased heterozygosity of the chromosomal segments marked by these enzyme loci and not the loci themselves. Linkage disequilibrium between the marker enzyme loci and any other loci having a heterotic effect on asymmetry, similar to the effects seen in interstrain hybrids, would produce these results.

Distinguishing among these possibilities is difficult; it is possible that all three of these mechanisms may contribute to the observed effect. The third explanation is appealing because it solves the dilemma of trying to explain such a large phenotypic effect by so few loci. We believe that the appropriate null hypothesis is that

the observed relationship between heterozygosity and asymmetry is caused by linked chromosomal segments and not the enzyme loci themselves. This hypothesis should only be rejected after finding substantial evidence indicating that the effect is due to the loci themselves.

We have begun to approach this problem using null alleles at enzyme loci. Such alleles produce no detectable enzyme product and are expected to have different effects on asymmetry depending on whether the effect is due to the enzymes themselves or to linked chromosomal segments. Null allele heterozygotes should not have reduced asymmetry if the effect is due to the enzyme itself or to a cis-acting regulatory element because they are enzymatically "homozygous" (i.e., only one active form of the enzyme is present). However, null allele heterozygotes are heterozygous for the chromosomal segment and thus should still tend to be less asymmetrical if the effect is mainly due to the chromosomal segment.

Our preliminary results (Leary et al. 1983*b*) with one locus (*Ldh3*) indicate that null allele heterozygotes are actually more asymmetrical than homozygotes for the active allele. These results suggest that this enzyme locus itself (including any tightly linked regulators) is affecting asymmetry. If there is a chromosomal effect, it is apparently being masked by the effect of the *Ldh3* locus.

The effects of linked chromosomal segments are expected to be greater under conditions that increase the possibility of linkage disequilibrium. For example, any inbreeding or nonrandom mating in the population would increase linkage disequilibrium and enhance the possible effects of linked chromosomal segments. There is no evidence that the populations we have studied are not and have not been random mating (with the exception of the McConaughy strain, see Results). There is no evidence of a Wahlund effect in genotypic proportions at individual loci or of linkage disequilibrium among the loci studied. In addition, the two rainbow trout populations showing a significant relationship between heterozygosity and asymmetry have been maintained in hatcheries under random mating for many generations.

Reduced recombination rates will also act to increase linkage disequilibrium. Salmonid males show greatly reduced rates of recombination in comparison with females (May et al. 1982). There is another feature of the salmonid genetic system that may increase linkage disequilibrium. Study of recombination between enzyme loci and their centromeres with gynogenetic diploid rainbow trout has revealed that very strong interference is apparently common (Thorgaard et al. 1983). In fact, for three nonsyntenic loci (*Sod1, Mdh3,* and *Mdh4*), there was complete or nearly complete interference so that there was one and only one crossover between the locus and its centromere. Thus, apparently there is reduced recombination in distal chromosomal segments. Linkage disequilibrium may, therefore, accumulate in these chromosomal segments bringing about heterotic effects similar to those seen in chromosomal inversions.

These ideas are admittedly speculative at present. However, there is suggestive evidence that this possibility is worth considering. Enzyme loci in rainbow trout that are far from their centromeres tend to be more polymorphic than proximal loci (F. W. Allendorf, R. F. Leary, K. L. Knudsen, and G. Thorgaard, unpubl. data). In addition, the loci in rainbow trout (table 2) with the most indication of

having an individual effect on asymmetry (*Sod1, Mdh3,* and *Mdh4*) are the same loci that are known to almost always have one and only one crossover in the interval between the locus and its centromere. In addition, heterozygosity at *Aat3,4* is significantly correlated with asymmetry in both populations of brook trout. Residual tetrasomic inheritance has been found to occur at these loci (Wright et al. 1980), and there is evidence that loci showing residual tetrasomic inheritance in salmonids are distant from their centromeres (Wright et al. 1983; Thorgaard et al. 1983).

Maintenance of Protein Polymorphisms

The association between heterozygosity and reduced fluctuating asymmetry combined with the association between fluctuating asymmetry and morphological deformities suggests that fitness is positively correlated with heterozygosity in these populations. Even if the effect is not due to these enzyme loci themselves, heterozygotes at these loci will be selected for through a hitchhiking effect. This suggests that variation at these loci is maintained, at least in part, by heterozygous advantage. This has implications for the debate about the forces maintaining genetic variation in natural populations. It is important to determine if the relationship between asymmetry and heterozygosity is in fact general or is for some reason peculiar to salmonid fishes that have a tetraploid ancestry.

SUMMARY

We examined the association between heterozygosity at 42 enzyme loci and fluctuating asymmetry at five bilateral meristic characters in 14 population samples of rainbow trout, cutthroat trout, and brook trout. There is a significant negative correlation between heterozygosity and the proportion of asymmetric characters per individual in two populations of rainbow trout and two populations of cutthroat trout. This correlation is negative in 13 of the 14 population samples (sign test, $P < .001$). Thus, individuals that are more heterozygous at isozyme loci have reduced fluctuating asymmetry; this appears to be general among salmonid fishes.

We also found that individuals with obvious morphological deformities in three populations have increased fluctuating asymmetry. Thus, fluctuating asymmetry appears to be a reliable indicator of overall developmental stability and is therefore negatively correlated with fitness. The association between heterozygosity and asymmetry and between asymmetry and morphological deformities suggest that heterozygosity is positively correlated with fitness in these fishes.

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